March 2004

Environmental Technology Verification Report

BIOQUELL, INC.
CLARUS C HYDROGEN PEROXIDE GAS GENERATOR

Prepared by
Battelle

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ETV ETV ETV

March 2004

Environmental Technology Verification Report

ETV Building Decontamination Technology Center

BIOQUELL, Inc.
CLARUS C Hydrogen Peroxide Gas Generator

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Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technologies across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of seven environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. In 2002, EPA established the Building Decontamination Technology Center at Battelle. Battelle plans, coordinates, and conducts verification tests of decontamination technologies and reports the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center9.html.

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List of Abbreviations

ANOVA	analysis of variance
BDT	Building Decontamination Technology
BWD	Bare wood (pine lumber)
CFU	colony-forming unit
cm	centimeter
DL	decorative laminate
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
GM	galvanized metal ductwork
GS	glass
HEPA	high-efficiency particulate air
IC	industrial-grade carpet
in	in
PC	painted (latex, semi-gloss) concrete cinder block
ppm	part per million
PW	painted (latex, flat) wallboard paper
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
SD	standard deviation
TSA	technical systems audit

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The EPA's National Risk Management Research Laboratory and its verification organization partner, Battelle, operate the Building Decontamination Technology (BDT) Center under ETV. The BDT Center recently evaluated the performance of the BIOQUELL, Inc., CLARUS™ C hydrogen peroxide gas generator for decontaminating buildings.

Chapter 2
Technology Description

The objective of the ETV BDT Center is to verify the performance characteristics of technologies that are designed to be used to decontaminate indoor surfaces in buildings contaminated with either chemical or biological agents as a result of an intentional attack or accidental release. This verification report provides results for verification testing of the CLARUS C unit. The following is a description of the CLARUS C unit, based on information provided by the vendor. The information provided below was not verified in this test.

The CLARUS C unit is a hydrogen peroxide gas generator (Figure 2-1) that uses a dual circuit system. The first circuit provides high-efficiency particulate air (HEPA) filtration, dehumidification, and hydrogen peroxide removal from the air stream via catalytic conversion. The second circuit delivers high-concentration hydrogen peroxide and water vapors. During gassing, the CLARUS C unit recirculates the vapors through the second circuit, constantly increasing the concentration of hydrogen peroxide and water vapor within the chamber or area intended for decontamination. This recirculation and vapor injection continues until the chamber reaches saturation, and the process of microcondensation begins. In microcondensation, a microscopic film of aqueous hydrogen peroxide solution is deposited on all surfaces. Once the gassing phase has been completed, the CLARUS C unit returns to the first circuit and brings the chamber to a safe condition by catalytically converting the hydrogen peroxide to water (humidity) and oxygen. Excess humidity is removed via the refrigerant-based dehumidification plant. To ensure that all essential data are captured, the CLARUS C unit prints out all critical parameters recorded throughout the cycle. The CLARUS C unit has a personal computer connection for more in-depth cycle analysis.

Figure 2-1. BIOQUELL, Inc. CLARUS™ C

The CLARUS C unit was designed to decontaminate enclosures of up to 7,000 cubic feet (200 cubic meters). It weighs 300 pounds (128 kilograms), and is 26 in (68 cm) wide by 35 in (90 cm) in depth by 45 in (106 cm) in height. The dehumidification system is designed to run continuously. Because there is no need for dehumidification regeneration down-time, the CLARUS C unit can operate continuously, if required, from a normal domestic power supply. The CLARUS C unit is controlled by a Siemens programmable logic controller, which is complemented by optional sensors (including a microcondensation sensor), allowing repeatable validated decontamination cycles.

For this verification test, the CLARUS C unit was attached to a Plas-Labs compact glove box modified according to the vendor's instructions (see Section 3.5.4.1). The CLARUS C unit and the glove box were connected by flexible supply and delivery gassing hoses that were HEPA-filtered. A hydrogen peroxide sensor, relative humidity sensor, and pressure sensing tube also were connected to the inside of the glove box, and data were transmitted through the glove box wall to the CLARUS C unit.

Chapter 3
Test Design and Procedures

3.1 Introduction

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Hydrogen Peroxide Vapor Technologies for Decontaminating Indoor Surfaces Contaminated with Biological or Chemical Agents.*[(1)] The biological and chemical agents that pose a threat to buildings include toxic industrial chemicals, chemical warfare agents, and biological warfare agents (including biotoxins). The biological agent selected for this verification test was *Bacillus anthracis* (Ames strain). In addition, two biological surrogates were used: *B. subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980). Seven materials representing indoor surfaces commonly found in buildings were used for the verification testing. The indoor surfaces tested (Figure 3-1) include

- Industrial-grade carpet (IC)
- Bare wood (pine lumber) (BWD)
- Glass (GS)
- Decorative laminate (DL)
- Galvanized metal ductwork (GM)
- Painted (latex, flat) wallboard paper (PW)
- Painted (latex, semi-gloss) concrete cinder block (PC).

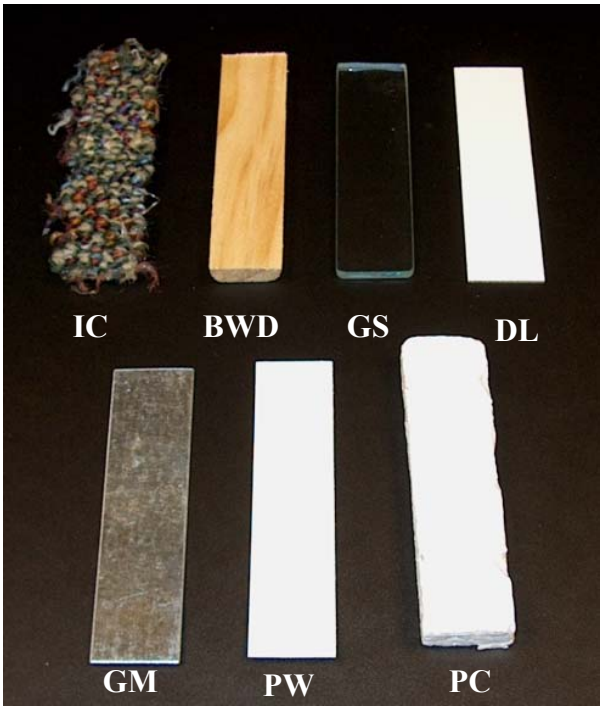

Figure 3-1. Test Materials

The objective of the verification testing was to evaluate the efficacy of the CLARUS C unit to decontaminate a biological agent/surrogate. Efficacy was tested by applying a biological agent and surrogates to the surfaces of test coupons and, after using CLARUS C, comparing the number of viable spores on decontaminated and control (non-decontaminated) samples. Visual inspection of the physical integrity of the test materials was performed, and

observations were recorded before and after implementing the CLARUS C unit technology in an effort to detect any degradation or chemical destruction of the material itself.

3.2 Test Design

Coupons were cut from larger pieces of the representative materials for each of the seven indoor surfaces (Section 3.1), measuring 3/4 x 3 in (1.9 x 7.5 cm) and having varying thickness from about 1/32 in (0.79 cm) to 3/8 in (0.95 cm), depending upon the material. In triplicate, the coupons were placed into a biological agent safety hood, and aliquots of an aqueous suspension of the biological agent were added to the surface of each coupon. Based upon the concentration of the spores in the aqueous suspension, the number of spores added to each coupon was calculated. The coupons were allowed to dry overnight. After drying, the inoculated coupons intended for decontamination were transferred into a custom-modified glove box and placed horizontally on a wire rack. Both blank (uncontaminated; N=2) and control (inoculated with spores, but not decontaminated; N=3) coupons were prepared, together with the inoculated coupons that were to be decontaminated (N=3).

Efficacy of the decontamination technology was determined by comparing the number of viable spores on the control coupons (not decontaminated) to the number present on the decontaminated coupons, expressed as a log reduction. Following extraction of spores from the test, control, and blank coupons, efficacy was further evaluated for each biological agent/surrogate by transferring each coupon into liquid growth medium and assessing bacterial growth after 1 and 7 days.

Physical degradation of the indoor materials used as test surfaces was evaluated informally in conjunction with the efficacy testing procedure. After decontaminating the test coupons, the appearance of the decontaminated coupons was observed; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were noted.

3.3 Agents and Surrogates

The following biological agent was used for verification testing:

- *Bacillus anthracis* spores (Ames strain).

To provide correlations with the biological agent results, two biological surrogates also were used:

- *Bacillus subtilis* spores (ATCC 19659)
- *Geobacillus stearothermophilus* spores (ATCC 12980).

Biological indicators and spore strips that were used to evaluate decontamination efficacy included:

- Biological indicators, approximately 10^6 spores each: *Bacillus subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980) spores on steel sealed in Tyvek® pouches

- Spore strips: with *Bacillus atrophaeus* (ATCC 9372) spores, approximately 10^6 spores per strip, on a filter paper matrix in sealed glassine envelopes.

3.4 Test Sequence

In Table 3-1, a summary of the verification testing of the the CLARUS C unit is presented. Verification testing was performed during a 10-week period that commenced in September 2003 and concluded in November 2003.

Table 3-1. Test Sequence and Parameters

Test Procedure	Parameters Evaluated	Data Produced
Biological Efficacy Test	Enumerations	Log reduction
	B. anthracis	
	B. subtilis	
	G. stearothermophilus	
	Liquid culture assessment of coupons	Positive/negative bacterial growth (1 and 7 days)
	B. anthracis	
	B. subtilis	
	G. stearothermophilus	
	Biological indicators/spore strips	Positive/negative bacterial growth (1 and 7 days)
	B. subtilis	
	G. stearothermophilus	
	B. atrophaeus	
Coupon Damage	Damage to test coupons	Visual observation of every test coupon in all biological efficacy tests before and after decontamination

3.5 Coupon-Scale Testing

Coupon-scale testing was used to evaluate the decontamination efficacy of the CLARUS C unit by extracting and measuring the viable biological spores on test coupons.

3.5.1 Preparation of Test Materials

Coupons used for biological agent decontamination were cut to about 3/4 x 3 in (1.9 x 7.5 cm) and prepared as shown in Table 3.2 by Battelle staff. Test coupons were visually inspected, and the condition of each coupon was recorded. The length, width, and thickness of the test coupons were measured and recorded. Chain-of-custody forms were used to ensure that the test coupons were traceable throughout all phases of testing.

Table 3-2. Material Characteristics

Material	Lot, Batch, or ASTM No., or Observation	Manufacturer /Supplier Name	Approximate Coupon Size, L x W x Thick, in	Material Preparation
Decorative Laminate	Laminate/ Formica/ White Matte Finish	Solid Surface Design	3 x 3/8	Wiped with 70% isopropanol
Galvanized Metal Ductwork	Industry HVAC standard 24 Gauge Galvanized Steel	Accurate Fabrication	3 x 3/8 x 0.0234	Cleaned with Acetone; wiped with 70% isopropanol
Glass	C1036	Brooks Brothers	3 x 3/8 x 1/8	Cleaned with Acetone; wiped with 70% isopropanol
Industrial-grade Carpet	ShawTek, EcoTek 6	Shaw Industries, Inc	3 x 3/8	Wiped with 70% isopropanol
Concrete, Cinder Block	ASTM C90	Wellnitz	3 x 3/8 x 3/8	Brush and roller painted all sides. One coat Martin Senour latex primer (#71-1185) and one coat Porter Paints latex semi-gloss finish (#919); wiped with 70% isopropanol
Wallboard Paper	05-16-03; Set-E-493; Roll-3	United States Gypsum Company	3 x 3/8	Roller painted on one side using Martin Senour Paints. One primer (#71-1185) and two finish (flat, #70-1001) coats; wiped with 70% isopropanol
Wood	Screen Molding (Pine Wood)	Kingswood Lumber	3 x 3/8 x 1/4	Wiped with 70% isopropanol

3.5.2 Application of Agents to Test Coupons

Biological agent test coupons were laid flat in a Biological Safety Cabinet (BSC) Class III and contaminated at challenge levels of approximately 1 x 10^8 spores per coupon. Working stock suspensions of the spores at the required concentration were prepared, transferred to the coupon using a micropipette, placing the suspension over the surface as small droplets. After contamination with biological agent or surrogate suspension, the test coupons were allowed to dry overnight, undisturbed. The next day, the inoculated test materials intended for decontamination (and one blank) were transferred to an isolator glove box that was

attached to the CLARUS C unit (see Section 3.5.4.1). The control inoculated test materials (not intended for decontamination) and one blank were left undisturbed in the BSC II.

3.5.3 Confirmation of Surface Applications

To confirm the application density of biological agents and surrogates, the *B. anthracis* and surrogate spore suspensions used to contaminate the coupons were re-enumerated on each day of use. This enumeration was carried out as described in Section 3.5.4.2.

3.5.4 Decontamination

3.5.4.1 Verification Testing Apparatus and Parameters

A Plas-Labs Compact Glove Box (Model 830-ABC, modified according to BIOQUELL's specifications (Figures 3-2 and 3-3 A-C), was used as the test chamber. The Plas-Labs Compact Glove Box is 28 in (71 cm) wide by 23 in (59 cm) in depth by 29 in (74 cm) in height and has a volume of 11.2 cubic feet (317 liters). The BIOQUELL unit was connected to the test chamber. The cycle parameters specified by BIOQUELL to be used for the testing were as follows:

- Cycle pressure: 20 Pascals
- Conditioning time: 10 minutes
- Gassing time: 20 minutes
- Gassing dwell: 20 minutes
- H_2O_2 injection rate: 2.0 grams per minute
- H_2O_2 dwell rate: 0.5 grams per minute
- Aeration time: set for 9,999 minutes.

Temperature parameters were not specified, and the CLARUS C unit did not measure temperature. Time, pressure, relative humidity, and hydrogen peroxide concentrations are monitored by the CLARUS C unit. Data collected with respect to these parameters can be printed by the CLARUS C unit. Using the specified cycle parameters, operation of the CLARUS C unit resulted in condensation on the surfaces inside the compact glove box (Figure 3-3 D).

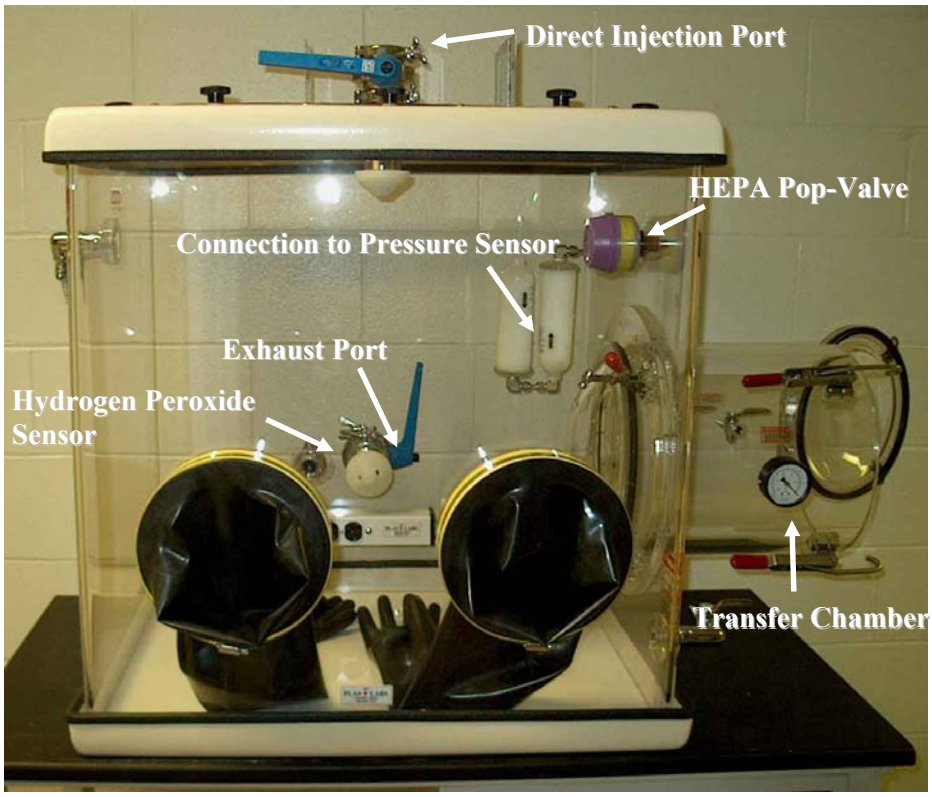

Figure 3-2. Overview of Plas-Labs Compact Glove Box Modifications

3.5.4.2 Decontamination Efficacy

Biological agent/surrogate decontamination efficacy was quantified by measuring the viable spores on both exposed (test) and unexposed (control) coupons. Each coupon was placed in a 50-milliliter (mL) test tube containing 10 mL of sterile phosphate-buffered saline to which 0.1% Triton X-100 and ~210 micrograms of catalase had been added. The purpose of the Triton X-100 was to minimize clumping of spores, and the purpose of the catalase was to neutralize residual hydrogen peroxide. For spore extraction, the tubes were agitated on an orbital shaker for 15 minutes at room temperature. Each tube was then heat-shocked at 60 - 65ºC for one hour to kill vegetative bacteria. Following heat-shock, 1.0 mL of each extract was removed, and a series of dilutions through 10^{-7} were prepared in sterile water.

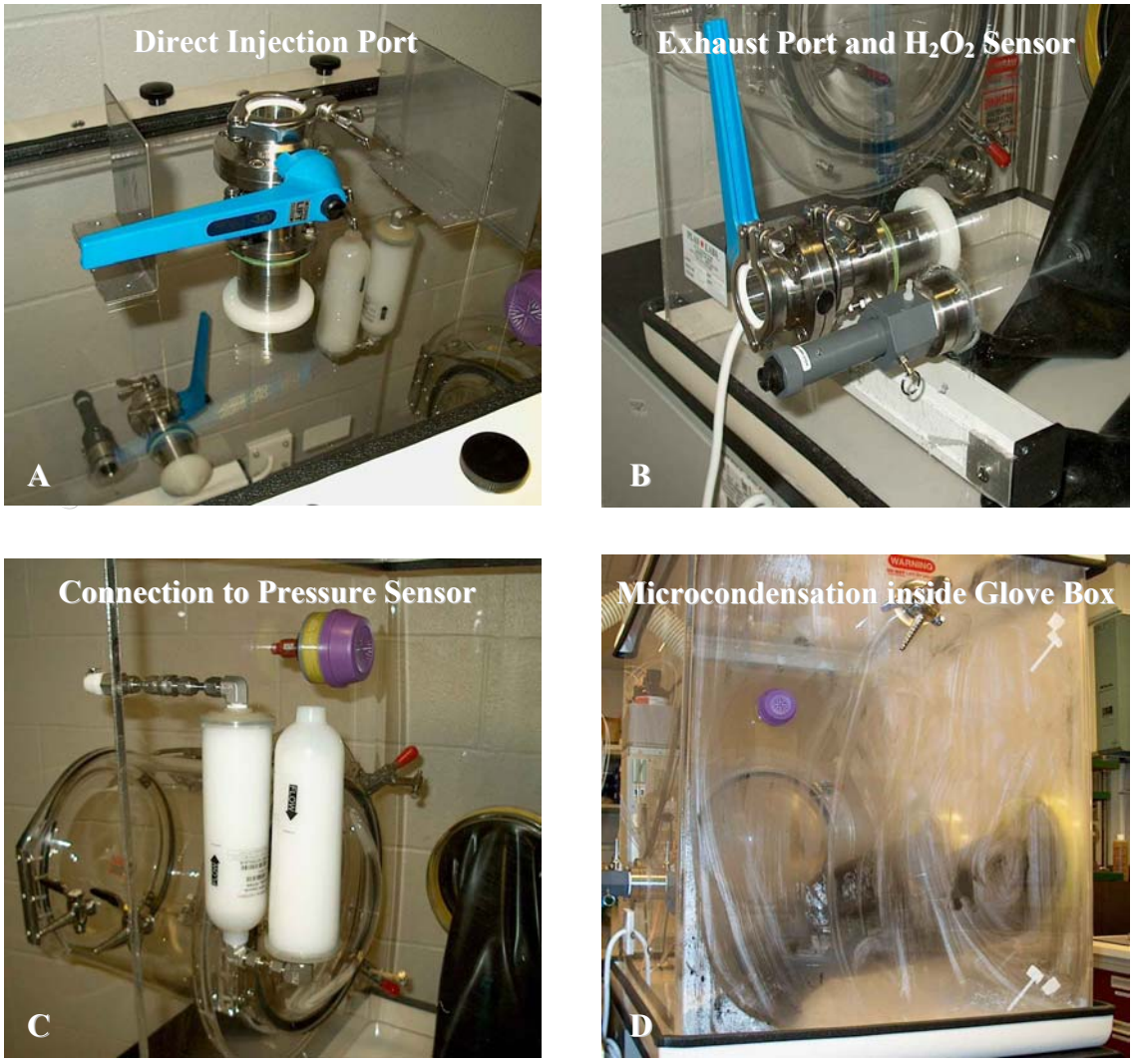

Figure 3-3. Detailed Views of Plas-Labs Compact Glove Box Modifications (A-C) and Condensation on Surfaces Within the Compact Glove Box (D)

Spore viability was determined by dilution plating, using both the undiluted extracts and the successive dilutions of each extract. One hundred microliters of the undiluted extract and of each serial dilution were plated onto tryptic soy agar plates in triplicate, allowed to dry, and incubated overnight at 35 to 37ºC for *B. anthracis* and *B. subtilis* and at 55 to 60ºC for *G. stearothermophilus*. Plates were enumerated the next day, and the colony-forming units (CFU)/mL were determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Data were expressed as a mean ± standard deviation (SD) of the number of CFUs observed. To calculate the efficacy of the decontamination treatment, the number of spores remaining on the decontaminated test coupons was compared to the number of spores on the control coupons. Efficacy for biological agents was expressed in terms of a log reduction.

An additional qualitative assessment of the CLARUS C unit efficacy was conducted following spore extraction. After the extraction process described above, each coupon was transferred to a sterile 50-mL tube containing 20 mL of tryptic soy broth culture medium. The vials were sealed and incubated on an orbital shaker at the appropriate temperatures (see above) for each organism. At 1 and 7 days post-decontamination, the tubes were visually assessed qualitatively for viability as "growth" or "no growth." The biological indicators and spore strips were also evaluated at 1 and 7 days post-decontamination for "growth" or "no growth."

3.5.5 Observation of Surface Damage

Following decontamination, each test surface was examined visually to establish whether decontamination using the CLARUS C unit caused any obvious damage to the surface. The coupons were observed immediately after completing the decontamination process, but before post-decontamination sampling. The surface was inspected by comparing the decontaminated test surface with control coupons of the same test material. Differences in color, reflectivity, contrast, and roughness were assessed and recorded.

Chapter 4
Quality Assurance/Quality Control

QA/quality control (QC) procedures were performed in accordance with the Quality Management Plan (QMP) for the BDT Center[2] and the test/QA plan for this verification test.[1] QA/QC procedures and results are described below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, Biosafety cabinets, etc.) used at the time of testing was verified as being certified, calibrated, or validated.

4.2 Audits

Two types of audit were performed during the verification test: a technical systems audit (TSA) of the verification test performance and an audit of data quality. Audit procedures are described below.

4.2.1 Technical Systems Audit

The Battelle Quality Assurance Unit conducted a TSA on September 10, 2003, to ensure that the verification test was being conducted in accordance with the test/QA plan[1] and the BDT Center QMP.[2] As part of the TSA, test procedures were compared to those specified in the test/QA plan, and data acquisition and handling procedures were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle Verification Test Coordinator for response. None of the findings of the TSA required corrective action. TSA records are permanently stored with the Quality Assurance Manager.

The EPA Quality Manager for the BDT Center conducted a TSA on September 10-11, 2003. A final TSA report from the EPA was received by Battelle on October 27, 2003. Battelle responded to the TSA finding, and submitted a final response to the TSA report on November 19, 2003. On November 28, 2003, it was noted by the EPA Quality Manager that Battelle's responses to findings were acceptable and that the audit was complete.

4.2.2 Audit of Data Quality

At least 10% of the data acquired during the verification test were audited. A Battelle Quality Assurance Officer traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.3 QA/QC Reporting

Each audit was documented in accordance with Section 3.3.4 of the QMP for the ETV BDT Center.[2] Once the audit reports were prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. A Battelle Quality Assurance Officer ensured that follow-up corrective action was taken. The results of the TSA were submitted to the EPA.

4.4 Data Review

Records generated in the verification test received a QC/technical review and a QA review before they were used to calculate, evaluate, or report verification results. Table 4-1 summarizes the types of data recorded and reviewed. All data were recorded by Battelle. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

Table 4-1. Summary of Data Recording Process

Data to Be Recorded	Where Recorded	How Often Recorded	Disposition of Data
Dates, times of test events	Data forms	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated into spreadsheets as necessary
Test parameters (agent/surrogate identities, concentrations, test surfaces, test conditions, etc.)	Data forms	When set or changed, or as needed to document the sequence of test	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sampling data	Data forms	At least at start/end of reference sample, and at each change of a test parameter	Used to organize/check test results; manually incorporated into spreadsheets as necessary
Biological enumeration and liquid culture assessment, chain of custody, and results	Data forms	Throughout sample handling and analysis process	Transferred to spreadsheets
Records and observations on CLARUS C unit use	Printout from the CLARUS C unit; data forms	Throughout implementation of the CLARUS C unit	Reviewed and summarized to support data interpretation
Surface damage	Data forms	Start/end of test	Used to assess damage of test materials following use of the CLARUS C unit

Chapter 5
Statistical Methods

The statistical methods for evaluating the efficacy of the CLARUS C unit are presented in this chapter. Qualitative observations also were used to evaluate verification test data.

5.1 Efficacy Calculations

For biological agents and surrogates, decontamination efficacy was calculated as the log reduction in viable organisms achieved by the CLARUS C unit. The efficacy (E), or log reduction, for the biological agent, or surrogates was calculated as

$$E = \log (N°/N)$$

where $N°$ is the mean number of viable organisms applied to the control coupons (i.e., those not subjected to decontamination), and N is the number of viable organisms recovered from each test coupon after decontamination. For decontaminated samples where viable organisms were not detected, the efficacy was calculated as the log of the mean number of viable organisms on the control coupons. Using the calculated log reduction for each test coupon, the mean log reduction (efficacy) ± SD was calculated.

Percent recovery was calculated for each type of test material inoculated with each biological agent/surrogate. Percent recovery (mean ± SD) was calculated by dividing the number of biological organisms in the treated sample by the number of biological organisms in the controls (non-decontaminated).

5.2 Statistical Analysis

For each material and species combination, log reduction was calculated as described above, resulting in a total of 63 log reduction values. In cases where no viable colonies remained after decontamination, one colony was assumed to be present for the purpose of this calculation. A two-way analysis of variance (ANOVA) model with main effects for *Bacillus* species and test material and interactions was fitted to the log reduction data. This model was used to compare each mean to zero, compare each surrogate to *B. anthracis* (within material) and compare each surrogate to *B. anthracis* for porous and non-porous materials. T-tests or statistical contrasts were used for the comparisons, with no adjustment for

multiple comparisons. The ANOVA model was fitted using the SAS® (version 8.2) GLM procedure.

Chapter 6
Test Results

The results of the verification test of the CLARUS C unit are presented in this section.

6.1 Efficacy

6.1.1 Bacillus anthracis *Ames Spores*

Exposure of material test coupons contaminated with *B. anthracis* Ames spores to the CLARUS C unit, using the vendor's specified parameters (Section 3.5.4), resulted in decontamination that varied according to the type of the test material (Table 6-1). The mean log reduction of detectable viable *B. anthracis* Ames spores ranged from 3.01 to 7.92 across all seven test materials. Three of these test materials (IC, BWD, PC) can be considered porous (on the inoculated surface), while the other four test materials (GS, DL, GM, PW) can be considered non-porous (on the inoculated surface). Based on the results for two of the porous materials, IC and BWD, decontamination of *B. anthracis* Ames spores from porous materials using the unit may be less effective than decontamination of non-porous materials. The log reduction in viable spores detected on the porous materials was 3.01, 3.70, and 6.36 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was 7.92, 7.85, 7.54, and 6.92 for GS, DL, GM, and PW, respectively.

A liquid culture growth assessment at 1 and 7 days post-decontamination was performed to determine whether viable *B. anthracis* Ames spores remained on the test materials following the extraction step (Table 6-2). The extraction efficiency for spores on all seven test materials was less than 100%; therefore, it was assumed that viable spores could remain on the test materials. Each test material was wiped with 70% isopropanol prior to inoculation (or non-inoculated blanks) with *B. anthracis* Ames spores; however, this isopropanol wash does not guarantee sterility, especially with the porous materials. The test materials were not autoclaved, due to some of the materials being damaged during the autoclaving process. Therefore, to maintain equivalent treatment/handling of the test materials, a 70% isopropanol wipe was used. The liquid culture assessment was intended to detect spores that remained on the test material following the extraction step; however, since the materials were not sterilized by autoclaving, this type of assessment may not discriminate between the growth of *B. anthracis* and/or other bacteria.

Table 6-1. CLARUS C Decontamination of *Bacillus anthracis* Ames Spores[a]

Test Material	Inoculum	Total Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	1.15×10^8	$6.87 \pm 0.32 \times 10^7$	59.7 ± 2.79	-[b]
Decontaminated	1.15×10^8	$9.29 \pm 7.23 \times 10^4$	0.081 ± 0.063	3.01 ± 2.11 (2.62-3.55)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare Wood (BWD)				
Control	1.07×10^8	$9.61 \pm 1.38 \times 10^6$	8.98 ± 1.29	-
Decontaminated	1.07×10^8	$3.30 \pm 2.87 \times 10^3$	0.0031 ± 0.0027	3.70 ± 0.67 (3.20-4.46)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	1.12×10^8	$8.41 \pm 2.18 \times 10^7$	75.1 ± 19.5	-
Decontaminated	1.12×10^8	0	0	7.92 ± 0 (7.92)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	1.15×10^8	$7.04 \pm 1.00 \times 10^7$	61.3 ± 8.71	-
Decontaminated	1.15×10^8	0	0	7.85 ± 0 (7.85)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)	1.12×10^8	$3.49 \pm 0.13 \times 10^7$	31.2 ± 1.12	-
Control	1.12×10^8	0	0	7.54 ± 0 (7.54)
Decontaminated	0	0	0	-
Blank (control)	0	0	0	-
Blank (decontaminated)				
Painted Wallboard Paper (PW)				
Control	1.07×10^8	$8.25 \pm 0.63 \times 10^6$	7.71 ± 0.59	-
Decontaminated	1.07×10^8	0	0	6.92 ± 0 (6.92)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	1.15×10^8	$3.79 \pm 1.68 \times 10^7$	32.9 ± 14.6	-
Decontaminated	1.15×10^8	$1.51 \pm 2.62 \times 10^3$	0.0013 ± 0.0023	6.36 ± 2.11 (3.92-7.58)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-

[a]Data are expressed as mean (± SD) total number of spores, percent recovery, and efficacy (log reduction). The efficacy range is shown in parentheses.
[b]Not Applicable

Table 6-2. Liquid Culture Growth Assessment of *Bacillus anthracis* Ames Spores

Test Material		Day 1				Day 7			
		S1	S2	S3	Bl	S1	S2	S3	Bl
Industrial-Grade Carpet (IC)	Control	-	-	-	-	-	-	-	-
	Decontaminated	-	-	-	-	-	-	-	-
Bare Wood (BWD)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	+	-	-
Glass (GS)	Control	+	+	+	-	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Decorative Laminate (DL)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	+	+	+	-
Galvanized Metal Ductwork (GM)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Painted Wallboard Paper (PW)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Painted Concrete (PC)	Control	-	-	-	-	+	+	+	-
	Decontaminated	-	-	-	-	-	+	+	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
Bl = Blank (not inoculated with *B. anthracis* Ames spores)
"+" = growth; "-" = no growth

Following the extraction step, each test coupon was placed into liquid culture to promote spore germination, thereby enabling the vegetative bacteria to proliferate. Positive growth was determined if the liquid culture medium turned cloudy, while no growth was determined when the liquid medium remained clear.

All of the liquid culture samples for IC (both control and decontaminated) were negative for bacterial growth. The brand of IC used for this test contains a product known as FlorSept®, which is considered a broad spectrum antimicrobial that is effective against Gram-positive and Gram-negative bacteria, as well as mold and fungi. It appears that under the conditions employed for this verification test, the FlorSept® may not be sporicidal since viable *B. anthracis* Ames spores were extracted from the IC and cultured on tryptic soy agar plates. Therefore, it is possible that, in the liquid cultures, FlorSept® may inhibit growth of vegetative cells derived from germination of the *B. anthracis* Ames spores. This growth inhibition was not unique to *B. anthracis*, as these results were also observed for *B. subtilis* and *G. stearothermophilus* (see Tables 6-5 and 6-8).

For all tests using *B. anthracis*, the control biological indicators and spore strips exhibited positive growth in the liquid cultures at both 1 and 7 days. No growth in the liquid cultures was observed at 1 and 7 days for the biological indicators and spore strips subjected to hydrogen peroxide exposure using the CLARUS C unit. A representation of the data from a single test day is shown in Table 6-3.

Table 6-3. Representative Liquid Culture Growth Assessment of Biological Indicators/Spore Strips

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	-	-	-	-	-
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Decontaminated	-	-	-	-	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

6.1.2 Bacillus subtilis *(ATCC 19659) Spores*

Exposure of test coupons contaminated with *B. subtilis* spores to the CLARUS C unit, using the vendor's specified parameters (Section 3.5.4), resulted in decontamination that varied according to the type of test material (Table 6-4). The log reduction of detectable viable *B. subtilis* spores ranged from approximately 1.63 to 7.66 for all seven test materials. Based on the results for two of the porous materials, IC and BWD, it appears that decontamination of *B. subtilis* (ATCC 19659) spores by the CLARUS C unit was less effective for porous materials than for non-porous materials. The log reduction in viable spores detected on the porous materials was 1.63, 2.18, and 6.09 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was 7.57, 7.66, 6.44, and 7.52 for GS, DL, GM, and PW, respectively.

Table 6-4. CLARUS C Decontamination of *Bacillus subtilis* Spores[a]

Test Material	Inoculum	Total Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	9.26×10^7	$4.69 \pm 0.19 \times 10^7$	50.7 ± 2.0	-[b]
Decontaminated	9.26×10^7	$1.16 \pm 0.42 \times 10^6$	1.25 ± 0.45	1.63 ± 0.15 (1.46-1.76)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare Wood (BWD)				
Control	9.26×10^7	$8.80 \pm 2.24 \times 10^5$	0.95 ± 0.24	-
Decontaminated	9.26×10^7	$8.06 \pm 6.11 \times 10^3$	0.0087 ± 0.0066	2.18 ± 0.50 (1.81-2.75)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	9.00×10^7	$3.71 \pm 2.03 \times 10^7$	41.3 ± 22.6	-
Decontaminated	9.00×10^7	0	0	7.57 ± 0 (7.57)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	9.00×10^7	$4.57 \pm 0.85 \times 10^7$	50.8 ± 9.49	-
Decontaminated	9.00×10^7	0	0	7.66 ± 0 (7.66)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	8.13×10^7	$3.62 \pm 0.76 \times 10^7$	44.5 ± 9.31	-
Decontaminated	8.13×10^7	3.33 ± 3.35 x 10	<0.0001	6.44 ± 0.98 (5.73-7.56)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard (PW)				
Control	8.13×10^7	$3.31 \pm 2.51 \times 10^7$	40.7 ± 30.8	-
Decontaminated	8.13×10^7	0	0	7.52 ± 0 (7.52)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	9.26×10^7	$1.26 \pm 0.16 \times 10^7$	13.6 ± 1.70	-
Decontaminated	9.26×10^7	2.20 ± 1.91 x 10	<0.0001	6.09 ± 0.88 (5.58-7.10)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-

[a]Data are expressed as mean (± SD) total number of spores, percent recovery, and efficacy (log reduction). The efficacy range is shown in parentheses.
[b]Not Applicable

A liquid culture growth assessment at 1 and 7 days post-decontamination was performed to determine whether viable *B. subtilis* spores remained on the test materials following the extraction step (Table 6-5). As stated above, each test material (or non-inoculated blank) was wiped with 70% isopropanol prior to inoculation with *B. subtilis* spores; however, this isopropanol wash does not guarantee sterility, especially with the porous materials. Therefore, positive growth observed in some of the test materials not inoculated with *B. subtilis* spores may have resulted from growth of other bacteria not affected by the 70% isopropanol wash.

Table 6-5. Liquid Culture Growth Assessment of *Bacillus subtilis* Spores

Test Material		Day 1				Day 7			
		S1	S2	S3	Bl	S1	S2	S3	Bl
Industrial-Grade Carpet (IC)	Control	-	-	-	-	-	-	-	-
	Decontaminated	-	-	-	-	-	-	-	-
Bare Wood (BWD)	Control	+	+	+	+	+	+	+	+
	Decontaminated	+	+	+	-	+	+	+	-
Glass (GS)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Decorative Laminate (DL)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork (GM)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Painted Wallboard Paper (PW)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Painted Concrete (PC)	Control	-	+	+	-	-	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
Bl = Blank (not inoculated with *B. subtilis* spores)
"+" = growth; "-" = no growth

For all tests using *B. subtilis*, the biological indicators and spore strips exhibited positive growth in the liquid cultures at both 1 and 7 days. No growth in the liquid cultures was observed at 1 and 7 days for the biological indicators and spore strips subject to hydrogen peroxide exposure using the CLARUS C unit. A representation of the data from a single test day is shown in Table 6-6.

Table 6-6. Representative Liquid Culture Growth Assessment of Biological Indicators/Spores Strips

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*B. subtilis* ATCC 19659)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*B. subtilis* ATCC 19659)	Decontaminated	-	-	-	-	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

6.1.3 Geobacillus stearothermophilus *(ATCC 12980) Spores*

Exposure of test coupons contaminated with *G. stearothermophilus* (ATCC 12980) spores to the CLARUS C unit, using the vendor's specified parameters (Section 3.5.4), resulted in decontamination, that varied according to the type of test material (Table 6-7). The log reduction of detectable viable *G. stearothermophilus* spores (ATCC 12980) ranged from approximately 0.81 to 5.98 for all seven test materials. The log reduction in viable spores detected on the porous materials was 0.81, 4.09, and 4.09 for IC, BWD, and PC, respectively. The log reduction in viable spores detected on the non-porous materials was 4.68, 3.75, 1.97, and 5.98 for GS, DL, GM, and PW, respectively. For *B. anthracis* Ames and *B. subtilis* (ATCC 19659) spores, porosity of the test material appeared to affect the decontamination efficacy of the CLARUS C unit. For *G. stearothermophilus* (ATCC 12980), there was no clear trend in decontamination efficacy between the porous and non-porous materials, as observed with *B. anthracis* and *B. subtilis*. Therefore, it is difficult to determine whether the porosity of the test materials influenced the decontamination efficacy of the CLARUS C unit. It appears that for one porous and one non-porous surface (IC and GM, respectively) there was a negative effect on the extent of decontamination of *G. stearothermophilis* (ATCC 12980) spores by the CLARUS C unit.

A liquid culture growth assessment at 1 and 7 days post-decontamination was performed to determine whether viable *G. stearothermophilus* spores remained on the test materials following the extraction step (Table 6-8). As stated above, each test material (or non-inoculated blank) was wiped with 70% isopropanol prior to inoculation with *G. stearothermophilus* spores; however, this isopropanol wash does not guarantee sterility, especially with the porous materials. Therefore, positive growth observed in some of the test materials not inoculated with *G. stearothermophilus* spores may have resulted from growth of other bacteria not affected by the 70% isopropanol wash.

Table 6-7. CLARUS C Decontamination of *Geobacillus stearothermophilus* Spores[a]

Test Material	Inoculum	Total Spores	% Recovery	Efficacy
Industrial-Grade Carpet (IC)				
Control	1.28×10^8	$2.69 \pm 0.051 \times 10^7$	21.0 ± 0.4	-[b]
Decontaminated	1.28×10^8	$4.28 \pm 1.08 \times 10^6$	3.34 ± 0.84	0.81 ± 0.10 (0.69-0.89)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Bare wood (BWD)				
Control	1.28×10^8	$2.76 \pm 0.081 \times 10^6$	2.15 ± 0.063	-
Decontaminated	1.28×10^8	$3.00 \pm 2.02 \times 10^2$	<0.001	4.09 ± 0.46 (3.80-4.61)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Glass (GS)				
Control	1.28×10^8	$8.72 \pm 0.58 \times 10^6$	6.82 ± 0.45	-
Decontaminated	1.28×10^8	$2.45 \pm 2.04 \times 10^2$	<0.001	4.68 ± 0.42 (4.27-5.11)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Decorative Laminate (DL)				
Control	9.50×10^7	$5.89 \pm 1.12 \times 10^6$	6.20 ± 1.18	-
Decontaminated	9.50×10^7	$1.26 \pm 2.14 \times 10^4$	0.013 ± 0.023	3.75 ± 1.37 (2.20-4.77)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Galvanized Metal Ductwork (GM)				
Control	1.28×10^8	$1.50 \pm 0.37 \times 10^7$	11.7 ± 2.89	-
Decontaminated	1.28×10^8	$1.64 \pm 0.27 \times 10^5$	0.13 ± 0.021	1.97 ± 0.07 (1.90-2.04)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Wallboard Paper (PW)				
Control	1.20×10^8	$9.73 \pm 0.81 \times 10^6$	8.11 ± 0.68	-
Decontaminated	1.20×10^8	$2.20 \pm 1.91 \times 10$	<0.0001	5.98 ± 0.88 (5.47-6.99)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-
Painted Concrete (PC)				
Control	1.20×10^8	$9.37 \pm 1.05 \times 10^6$	7.81 ± 0.87	-
Decontaminated	1.20×10^8	$2.85 \pm 4.11 \times 10^3$	0.0024 ± 0.0034	4.09 ± 1.03 (3.09-5.15)
Blank (control)	0	0	0	-
Blank (decontaminated)	0	0	0	-

[a]Data are expressed as mean (± SD) total number of spores, percent recovery, and efficacy (log reduction). The efficacy range is shown in parentheses.
[b]Not Applicable

Table 6-8. Liquid Culture Growth Assessment of *Geobacillus stearothermophilus* Spores

Test Material		Day 1				Day 7			
		S1	S2	S3	Bl	S1	S2	S3	Bl
Industrial-Grade Carpet (IC)	Control	-	-	-	-	-	+	-	-
	Decontaminated	-	-	-	-	-	-	-	-
Bare Wood (BWD)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	+	+	-	+	+	+	-
Glass (GS)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Decorative Laminate (DL)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork (GM)	Control	+	+	+	-	+	+	+	-
	Decontaminated	+	+	+	-	+	+	+	-
Painted Wallboard Paper (PW)	Control	+	+	+	+	+	+	+	+
	Decontaminated	-	-	-	-	-	-	-	-
Painted Concrete (PC)	Control	+	+	+	-	+	+	+	-
	Decontaminated	-	-	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
Bl = Blank (not inoculated with *G. stearothermophilus* spores)
"+" = growth; "-" = no growth

For all tests using *G. stearothermophilus*, the biological indicators and spore strips exhibited positive growth in the liquid cultures at both 1 and 7 days. No growth in the liquid cultures was observed at 1 and 7 days for the biological indicators and spore strips subject to hydrogen peroxide exposure using the CLARUS C unit. A representation of the data from a single test day is shown in Table 6-9.

6.1.4 Statistical Analysis

Table 6-10 presents the mean log reduction in spores sorted by material type. Significant differences are also indicated in the table. All means were significantly different from zero except for the mean log reduction of *G. stearothermophilus* for carpet, indicating that the technology decontaminated statistically significant numbers of spores on these materials.

Table 6-9. Representative Liquid Culture Growth Assessment of Biological Indicators/Spores Strips

Indicator (Organism)		Day 1			Day 7		
		S1	S2	S3	S1	S2	S3
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Control	+	+	+	+	+	+
Spore Strip (*B. atrophaeus* ATCC 9372)	Control	+	+	+	+	+	+
Biological Indicator (*G. stearothermophilus* ATCC 12980)	Decontaminated	-	-	-	-	-	-
Spore Strip (*B. atrophaeus* ATCC 9372)	Decontaminated	-	-	-	-	-	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
"+" = growth; "-" = no growth

Table 6-10. Statistical Analysis of Mean Efficacy (Log Reduction) for Spores

Material		*B. anthracis*	*B. subtilis*	*G. stearothermophilus*
Porous	**Industrial-Grade Carpet**	3.01 [a]	1.63 [a b]	0.81 [b]
	Painted Concrete	6.36 [a]	6.09 [a]	4.09 [a b]
	Bare Wood	3.70 [a]	2.18 [a b]	4.09 [a]
Non-Porous	**Glass**	7.92 [a]	7.57 [a]	4.68 [a b]
	Decorative Laminate	7.85 [a]	7.66 [a]	3.75 [a b]
	Painted Wallboard Paper	6.92 [a]	7.52 [a]	5.98 [a]
	Galvanized Metal Ductwork	7.54 [a]	6.44 [a]	1.97 [a b]

[a]Mean significantly different from 0 at the ($P \leq 0.05$)
[b]Surrogate significantly different from *B. anthracis* for specified material ($P \leq 0.05$).

For both IC and BWD, there appeared to be a negative effect on the degree of decontamination of *B. anthracis* and the two surrogates by the CLARUS C unit. Comparisons within each material indicated that *B. subtilis* had a significantly lower mean log reduction in spores from *B. anthracis* for IC and BWD. *G. stearothermophilus* had significantly lower mean log reductions in spores from *B. anthracis* for IC, PC, GS, DL, and GM. For two of the three porous materials, both *B. subtilis* and *G. stearothermophilus* had significantly different mean log reductions from *B. anthracis.* For non-porous materials, *G. stearothermophilus* was significantly different than *B. anthracis.* These overall results are consistent with the results for each material.

6.2 Damage to Coupons

Subsequent to decontamination, the test coupons were evaluated qualitatively for visible surface damage. No damage (e.g., change in surface texture, color, etc.) and no visible changes to any of the test materials were observed during this verification test.

6.3 Other Factors

6.3.1 Operation of the CLARUS C Unit

The CLARUS C unit was operated for approximately 160 hours during this verification test. By following the user manual, the CLARUS C unit can be set up and programmed for operation within minutes. The program containing defined test parameters can be stored, retrieved, and executed by the CLARUS C unit within seconds. The only maintenance required for the CLARUS C unit during this verification test was the addition of new hydrogen peroxide at the beginning of each run and disposal of unused hydrogen peroxide and waste by-product (i.e., water) at the end of each run. The printer paper had to be refilled once during testing.

Data for the cycle parameters (hydrogen peroxide concentration, relative humidity, and pressure) are monitored in real-time. The data are stored by a personal computer connected to the CLARUS C unit, as well as displayed on the control panel. A secondary record of the data is provided as a printout from the CLARUS C unit. The data on the printout consist of values from the start of the cycle and the start and completion of each phase. The data collected with respect to cycle parameters were acquired as the paper printout from the CLARUS C unit. As a representation of the data collected, Table 6-11 shows values from a single run. These values include pressure, relative humidity, and hydrogen peroxide concentration within the Plas-Labs Compact Glove Box, and were acquired as the paper printout from the CLARUS C unit.

For this verification test, cycle parameter data were not collected in real time by the personal computer that was provided with the CLARUS C unit. This was due to a non-functional networking card that is intended to enable communication between the CLARUS C unit and the personal computer. The networking card was replaced near the end of the verification test, and sample data were collected for the last two runs. Figure 6-1 is a graphical representation of the real-time measurements for hydrogen peroxide concentration, relative humidity, and pressure from 0 to 9 hours of an approximately 16-hour run. From the real-time data collected, beyond 9 hours, the hydrogen peroxide concentration and relative humidity were approximately 0 ppm and 0%, respectively, while the pressure was approximately 20 Pascals inside the Plas-Labs Compact Glove Box.

Table 6-11. Representative Data from CLARUS C Printout

Phase	Pressure (Pa)	Relative Humidity (%)	H_2O_2 Concentration (ppm)
Conditioning			
Start	0	25.2	0
Finish	20.4	41.4	0
Pre-Gassing			
Start	16.0	NR[a]	0
Finish	20.8	NR	0
Gassing			
Start	20.8	NR	0
Finish	14.5	NR	923.6
Gassing Dwell			
Start	14.5	NR	923.6
Finish	25.2	NR	1000.0
Aeration			
Start	25.2	NR	1000.0
Finish	8.9	NR	0

[a]NR = not reported on printout.

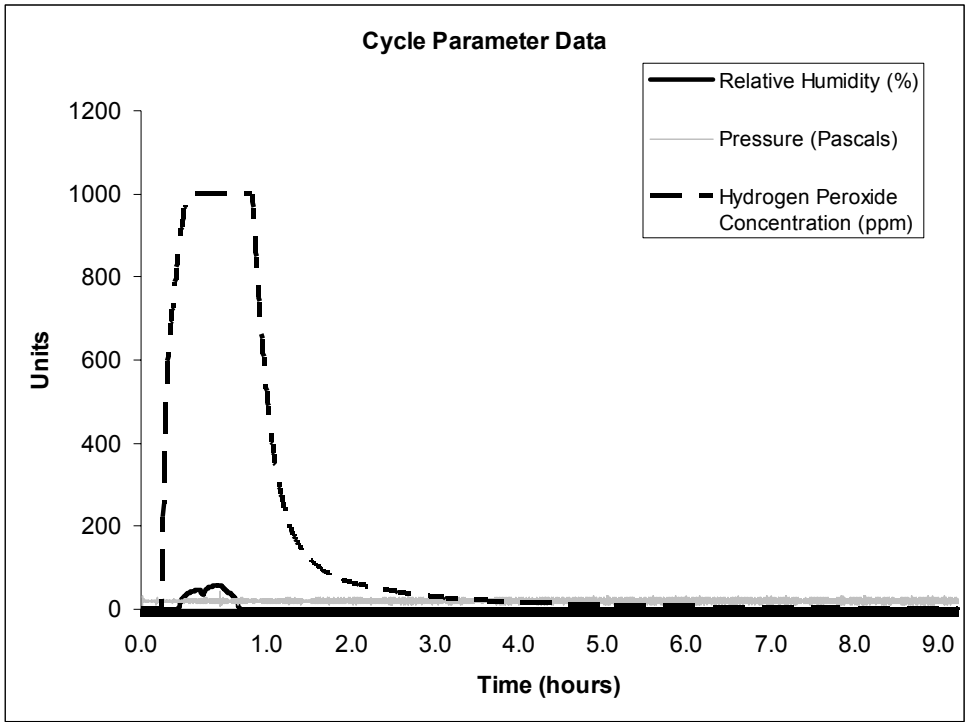

Figure 6-1. Representative Cycle Parameter Data from a Single Experiment

6.3.2 Operator Bias

Due to the automated capabilities of the CLARUS C unit, there is little room for operator error. The CLARUS C unit provides storage of specific cycle parameters, thereby enabling the user to turn on the machine, select the pre-programmed cycle parameters, and press start. The machine runs through the cycle and remains in the aeration phase (set for 9,999 minutes) until the machine is turned off. The aeration phase was allowed to run overnight and shut off the next morning; therefore, a total run time from start to finish was approximately 16 to 18 hours. By the end of the aeration phase, the hydrogen peroxide concentration was 0 ppm. During the run, the hydrogen peroxide concentration, relative humidity, and pressure was monitored and regulated by the CLARUS C unit, thereby preventing operator error associated with these parameters.

Chapter 7
Performance Summary

For this verification test, the CLARUS C unit demonstrated a range of decontamination efficacy for all three biological organisms on all seven test materials. Based on these results, different material types influence the efficacy of decontamination differently for various organisms. IC and BWD had the lowest level of *B. anthracis* Ames and *B. subtilis* (ATCC 19659) spore decontamination. However, for *G. stearothermophilus* (ATCC 12980), IC and GM resulted in the lowest decontamination efficacy. GM exhibited little or no impact on decontamination of *B. anthracis* Ames and *B. subtilis* (ATCC 19659).

The ETV testing to measure the effectiveness of the CLARUS C unit for inactivating *B. anthracis* Ames strain and surrogate spores on seven different indoor surfaces provides a range of results. A quantitative evaluation of the results indicates that the log reduction values for detectable viable *B. anthracis* Ames spores ranged from 3.01 to 7.92 across all seven test materials. The log reduction values for detectable viable *B. subtilis* spores ranged from 1.63 to 7.66 for all seven test materials. The log reduction values for detectable viable *G. stearothermophilus* spores (ATCC 12980) ranged from 0.81 to 5.98 for all seven test materials. In general, significant differences in efficacy between *B. anthracis* and both surrogate organisms were observed on porous materials. For non-porous materials, significant differences in efficacy between *B. anthracis* and *G. stearothermophilus* were observed in most cases.

A qualitative evaluation of the performance of the CLARUS C unit could be based upon the growth assessment of the biological indicators and spore strips. For all procedures for this verification test, the control (not exposed to the CLARUS C unit) biological indicators and spore strips used in this test displayed positive growth in the liquid cultures at both 1 and 7 days. When the biological indicators and spore strips were treated, no growth was observed in the liquid cultures at 1 and 7 days. Based on these results, the CLARUS C unit inactivated both the biological indicators (containing *B. subtilis* and *G. stearothermophilus*) and spores strips (containing *B. atrophaeus*), all of which contain spore loads of approximately 1×10^6 spores per indicator or spore strip.

The CLARUS C unit can be set up and programmed for operation within minutes. Data for the cycle parameters are monitored in real-time and stored/displayed via several mechanisms. During this verification test, cycle parameter data were not collected in real time by the personal computer that was provided with the CLARUS C unit. Therefore, the data collected with respect to cycle parameters were derived from the paper printout. Based

on the data from this printout, the CLARUS C unit appeared to operate within the test parameters provided by the vendor and programmed into it. The effect of operator skill level on using the CLARUS C unit, while not verified in this test, should be minimal due to its automated capabilities, which left little room for operator error.

Chapter 8
References

1. *Test/QA Plan for Verification of Hydrogen Peroxide Vapor Technologies for Decontaminating Indoor Surfaces Contaminated with Biological or Chemical Agents,* Battelle, Columbus, Ohio, July, 2003.

2. *Quality Management Plan (QMP) for the Technology Verification of Commercially Available Methods for Decontamination of Indoor Surfaces Contaminated with Biological or Chemical Agents,* Version 1, prepared by Battelle, Columbus, Ohio, November 22, 2002.

These references are posted on the ETV web site at:
http://www.epa.gov/etv/centers/center9.html